ESR 3 (WP1): Daria Kaptsan (d.kaptsan@vu.nl), Vrie University Amsterdam, the Netherlands

Local supervisory team: Dr Rob van Spanning, Dr Wilfred Röling, Professor Bas Teusink

Project: Modelling nitrous oxide emissions from cellular networks to environmental behaviour

This project will use system biology approaches, in conjunction with experimental work, to identify the factors controlling and regulating the N_2O emissions in denitrifying bacteria. We will develop kinetic models, parameterize them, and optimize them for describing growth and flux of the well-known denitrifier *Paracoccus denitrificans*. The practical part will, in part, include cultivation experiments, including cultivation in retentostats. This is a suitable tool to simulate the growth of microorganisms at low growth rate under natural conditions. Among other factors, the physiology of bacteria, gas emissions and gene expression under various nutrient limitations will be measured. The influence of metal-containing compounds on the denitrification and N_2O emission rates will be determined and growth model developed. A mixed culture model of *Paracoccus* mutants will be developed and experimentally tested in chemostat, in order to establish if the mutants can coexist, despite producing toxic components. It is also desirable to explore and model the denitrification capability of thermophilic gram positive bacteria like *Geobacillus kaustophilus*, which cannot perform the last step of the denitrification pathway, because of the absence of an N_2O reductase. Further emphasis will be on the interaction of denitrifiers and nitrifiers and consequences for nitrogen cycling. These community processes will be studied in retentostats and flux models will be generated.

Specific research goals of the project:

1. Development, parameterization and optimization of a kinetic model of denitrification of *P. denitrificans.*

It will be integrated with regulatory data.For that we will elucidate the role of *nos* gene regulation in relation to N_2O emissions. We have identified NNR, FnrP, NosR and RegAB as regulators of *nos* gene expression. The interplay between these regulators will be determined and effects on N_2O emissions will be modelled (secondment with UEA).

2. Determination of control of respiratory enzymes on flux and concentration of N2O. For that we aim at a quantitative description of the physiology and N2O production of denitrifying *Paracoccus denitrificans* at growth rates relevant for natural environments. This is to highlight the importance of growth rates on denitrification rates and N_2O emissions. Experiments will be carried out in chemo- and retentostats (secondment with TU Delft and UEA).

3. Development of community flux model to study effects of interacting species on flux and concentration of N_2O. The types of community include nitrifier-denitrifier, denitrifier-DNRA (dissimilatory nitrate to ammonium reduction. Retentostats will be used to culture stable communities growing at low growth rates (secondment with UMB).

4. N_2O production by thermophilic gram positive *Geobacillus kaustophilus.*Little is known about nitrate and nitrite fluxes in environments with high temperatures. *G. kaustophilus* research on denitrification my shed light on the physiology of these types of organism and their contribution to N_2O emissions in the atmosphere (secondment with UMB).